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Antimicrobial Surfaces of Metal Halides Immobilized on Polymeric Materials

Caicedo Carolina¹, Edwin Flórez López,¹ Vergara John, Guateque Jhon², Ávila-Torres Yenny^{1*}

¹ Grupo de investigación QUIBIO, Facultad de Ciencias Básicas, Universidad Santiago de Cali, Santiago de Cali, Pampalinda, Colombia.

² Grupo de investigación GIRAB, Universidad de Antioquia, Medellín, Colombia.

E-mail: yennytorres@usc.edu.co

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Abstract

Chitosan is a biopolymer extracted from the shell of crustaceans. It is currently possible that this be modified to improve its bacterial properties. In this context, in the present work new materials were synthesized as electro-spun films, where the best conditions were established that led to biocidal activity against *Staphylococcus aureus* ATCC 6538. The addition of PVA to chitosan improves electrospun conditions since the chitosan alone cannot stabilize the voltage. The best interaction with water was resolved at 16 % PVA, increasing the hydrophobic capacity of the film. The ion metallic interacts with chitosan matrix through acetyl group, and the alcohol and ammine group do not participate in electronic interaction. Finally, this work shows that the metal ion increases the biocidal properties of chitosan as a thin film.

Keywords: chitosan, electrospinning, antimicrobial activity

1. Introduction

Chitosan has been used as an antimicrobial agent in the following microorganisms: *Botrytis cinerea*, *Fusarium oxysporum*, *Drechtera sorokiana*, *Micronectriella nivallis*, *Piricularia orizae*, *Rhizoctonia solanii*, *Trichophyton equinum*, algae and some Gram positive and negative bacteria such as *Escherichia coli* (EC), *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*. The antimicrobial action is influenced by intrinsic factors such as chitosan type, molecular weight, culture medium pH, environmental conditions, host, chemical and nutrient composition of the substrate, degree of depolymerization and the presence or absence of lipids and proteins as interfere. Chitosan inhibitory activity versus phytopathogenic fungi has been observed between pH 6 - 7.5, which is corresponding with amino groups protonated. On the other hand, also is observed a relationship between the antimicrobial capacity of chitosan and the degree of deacetylation. The action mechanism of chitosan antibacterial activity of chitosan against Gram negative bacteria has shown an increase of the potassium ion released to the medium and a decrease of the bacterial count in the presence of chitosan [1-3]. The interaction of free amino group protonated with the cell wall, interferes with the barrier properties of the cell wall, causing loss of intracellular constituents and death of the bacterium. In the case of metal ions associated with antimicrobial activities, it has been reported that silver (Ag⁺) ions are a source of bacterial inhibitors. However, its use has been severely limited by the toxicity of ions in humans. In this sense, nanotechnology has allowed the production of small silver particles with an increasing surface area by volume, which has an efficacy against bacteria, and most importantly, a lower toxicity.



The metallic cations can be adsorbed by chelation on amine groups of chitosan in near-neutral solutions. Thorough of electronic pairs corresponding acetyl group and of the nitrogen is coordinated the chitosan towards metal ion. The pH is an important variable that discriminates coordination mode and electronic effects. Currently, these systems with Pd, Pt, Mo, V, Cu, Ag presents some applications such as: decontamination of effluents, optoelectronic applications, recovery of valuable metals, modification of antimicrobials, among others.

At the microbiological level, chitosan nanoparticles functionalized with anions such as *tripolyphosphate* generate a negative residual charge that electrostatically interacts with metal ions such as Cu^{2+} . These materials have shown antibacterial activity against *EC*, *S. choleraesuis*, *S. typhimurium* and *SA*. This activity suggests that chitosan disrupts cell membranes and cytoplasmic leakage. Likewise, the transcriptional analysis revealed that chitosan treatment leads to multiple changes in the expression profiles of SG511 *Staphylococcus aureus* genes. At the level of multilayer films of chitosan with nanosilver particles on **PET** (Polyethylene terephthalate), cytotoxic activity has also been reported. However, today the method of deposition of nanofibers, by electrospinning, has been used to introduce antibacterial activity and biocompatibility to the surface of PET textiles. Mixtures of PET and chitosan polymers are electro-passed to PET micro-non-woven mats for biomedical applications. The antibacterial activity of the samples evaluated against *Staphylococcus aureus* and *Klebsiella pneumoniae* have shown a significantly higher rate growth inhibition in relationship with **PET** nanofiber. In addition, fibroblast cells are better adherent to **PET**/ chitosan nanofibers than to **PET** nanofiber mats, suggesting better tissue compatibility.

Given the previous context, in the present article a report of **Q-PVA**, where PVA is polyvinyl alcohol, spinning via electrospinning on aluminum. Later its coordination to metallic centers of copper (II), cobalt (II), silver (I) and manganese (II). Finally, the antibacterial evaluation against *Staphylococcus aureus* ATCC 6538 with the modified Kirby - Bauer method on discs, comparing the different surfaces of **Q** with metal halides in relationship to the respective complexes in solution without spinning.

2. Experimental details

2.1. Physical measurements: The UV-Vis-NIR spectra (diffuse reflectance, $40000\text{--}4000\text{ cm}^{-1}$) were recorded on a Cary -5E (Varian) spectrophotometer.

2.2 Materials: $\text{CuCl}_2 \cdot \text{H}_2\text{O}$, CH_3COOH , $\text{CoCl}_2 \cdot 4\text{H}_2\text{O}$, MnCO_3 , AgCl, Chitosan MWL (Chitosan of low weight molecular), Chitosan MWM (Chitosan of medium weight molecular) Aldrich. Solvents: methanol, distilled water, Mueller-Hinton agar, Infusion-Brain-Heart (BHI).

2.3 Electrospinning method: The solutions prepared were supplied at room temperature in a syringe of diameter 12.45 mm, with needle (metal capillary), internal diameter 0.7 mm. The flow rate programmed in the injection pump (Syringe Pump, NE-1600) was 0.2 mL/h to 0.120 mL/h. A static aluminium plate was used to collect the electrospun nanofibers and eroded glass with HF at different distances (15, 16, 17, 18, 19, 20 and 21 cm). The applied voltage varied between 12 kV and 20 kV in which mixtures of PVA polymers and chitosan. For copper(II), cobalt(II) the solution were of pH 6.0 and manganese(II) pH=5.0 and silver(I) pH=4.0, **Table 1**.

Table 1. Electrospun parameters for Chitosan and metallic ions

Conditions	Parameters
Chitosan	QBPM, QMPM
Collector distance	11 cm
Pumping speed	0.07 -0.01 mL/h
Variable voltage	10, 12, 14, 16, 18 kV
Time	30, 45, 60, 70 min
% PVA	16%
Metallic ion 2%	Cu(II), Co(II), Mn(II), Ag(I)

2.4 Microbiological evaluation method: The preparation of the *Staphylococcus aureus* ATCC 6538 inoculum was initially carried out in the Infusion-Brain-Heart, incubating for a time of 48 h. Subsequently, sowing was done by depth, on the Mueller-Hinton agar, depositing impregnated discs of the mixtures in liquid state and films spun aluminum. Seeding was performed in triplicate in each scheduled experiment, with an incubation period of 24 h at 37 °C. The antibiotic used as control was reactive grade ampicillin, with a concentration of 5×10^5 ppm.

2.5. Preparation of solutions: The PVA solutions were prepared at 8% and 16% w/v with distilled water at 80 °C by constant stirring. Also, low molecular weight and medium molecular weight chitosan solutions were prepared at 2% acetic acid at room temperature. Solutions of 16% PVA and low molecular weight chitosan were mixed in different volume proportions (PVA-Q) 70:30, 80:20 and 90:10. These new solutions were stirred for 1 hour at room temperature. For metallic halide solutions, the 2% molecular weight chitosan solutions were co-solved with 16% polyvinyl alcohol at room temperature, pH 3.5, as water solvent and stirring for 30 min. After homogenization of the solutions, electrospinning was carried out, **Table 1**. For metallic salts the concentration were around of 2%.

2.6 Microstructural analysis: The fibers were analyzed through scanning electron microscopy (SEM). Using an SEM JEOL de Mesa JCM 50000. They were submitted to a gold coverage using PVD, in a high vacuum and a 10 kV voltage was used. The magnifications were x1000 to determine the dispersion, x10000 to assess the general morphology. It is worth to mention, that for every analysis the sample were conditioned to 25 °C in an environment of relative humidity of 50±5% during 48 h.

Contact Angle Analysis: Ramé-Hart Model 250 goniometer was used for this measurement with an optical system with which the interaction of water (2 µL) with the surface of the films of the samples was observed. The image was captured after 60 s and the analysis was carried out with Image J software. Three measurements were made for each sample to take an average of the measurements.

3. Results and discussion

3.1. Characterization of QMPM and QBPM solutions with PVA

Based on the results reported in **Table 2**, the use of QBPM with respect to QMPM increases the viscosity of the solutions, which suggests a higher degree of entanglement. As the content of PVA in the solutions increases the conductivity increases, since the PVA is soluble in water, contrary to what happens with the Q which must be dissolved in acidic medium (2% acetic acid). Also the groups -OH of the PVA can agglomerate exposing on the surface these hydrophilic groups, which does not necessarily imply that the viscosity drops. On the other hand, it is important to mention that the best electro-spinning conditions are based, on an average concentration value; where the polymer fibbers do not break into drops before reaching the collecting plane. This is due to the effect of surface tension by a low concentration of polymer; or difficulty in passing the solution through the capillary, due to a very concentrated and highly viscous solution, (Duque, 2013). At the spectroscopic level, shows the allocation of the characteristic vibrations of the chitosan in the presence of PVA at different concentrations. There is a lower energy shift of the vibration -(NH) and -(OH) depending on the increase in PVA, which suggests an increase in molecular hydrogen bond interactions for these groups. In order to obtain a better electro-spinning, the QMPM is chosen since it presented lower viscosity, which is a determining factor in obtaining nanofibers and prevents drip formation in the collector.

Table 2. Characterization of the Q and PVA

Concentration	Percent (%)	Viscosity (cP)	Conductivity (mS/cm)
PVA in QBPM	8	67.2	1.69
	16	708.0	2.20
	80	2068.0	2.36
	90	2115.1	2.48
PVA in QMPM	8	55.4	1.86
	16	620.4	2.48
	80	1985.7	2.57
	90	2064.2	2.68

3.2. Nanofibers from QMPW with PVA

The addition of polyvinyl alcohol to the chitosan matrix improves the quality of the electro-spinning. **Figure 1** shows the electron microscopy images for the different solutions made up of 8%, 16%, 80 and 90% PVA independently. The crosslinking between the polymer and the fiber is observed; which improves depending on the increase in PVA. However, in **Table 3**, the size distribution is reported; for the concentration around 16% the distribution is uniform (0.113-0.237 μm), which is why the concentration was selected to electro spin.

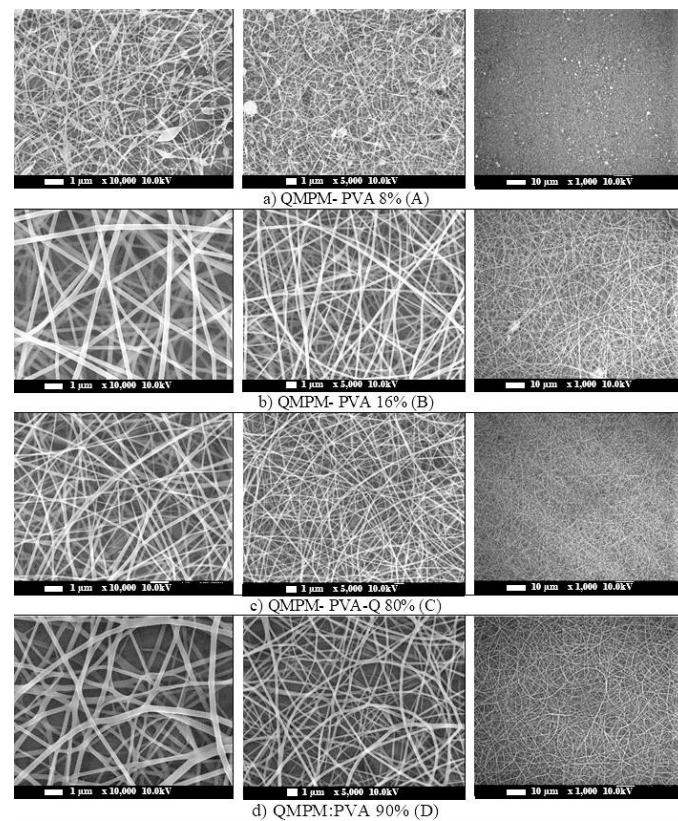
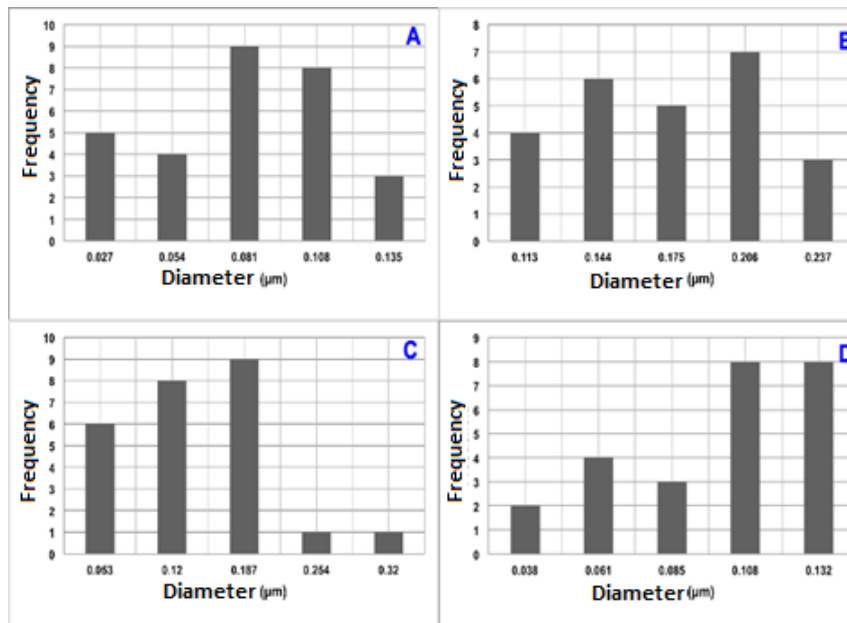
**Figure 1.** Micrographs of QMPM: PVA at different concentrations a) 8%, b) 16%, c) 80% and d) 90%

Table 3. Size distribution in the QMPM-PVA mixtures.

In the stabilization of nanofibers it is convenient to determine the interaction of water (6 μL) with the surface of the membranes, since the applications will be based on the solvent and stability in aqueous medium. In this sense, a Ramé-Hart goniometer with an optical system for QMPM-PVA at different concentrations was used in the measurement of the contact angle, table 6; then the image of the video was captured at the initial time t_0 (1 s) and final t_f (30 s), with Image J6 software. The contact angle shows the relationship between the adhesive forces between the liquid and the solid and cohesive forces of the liquid. The highest interaction with water was 16% QMPM-PVA, which is consistent with the characteristics of the chitosan solubilized in acetic acid with increased interaction due to the effect of hydrophilic areas of the PVA, **Table 4**.

Table 4. Contact angle for concentration different QMPM- PVA

Sample	Contact angle (°)
Q- PVA 16%	t_0 : 67.7; t_f : 67.2
Q- PVA 90%	t_0 : 55.1; t_f : 54.3
Q- PVA 80%	t_0 : 49.8; t_f : 45.5

Based on the above characterization, the concentration of QMPM: 16% PVA was chosen as the electrohilated solution. The conditions to be adjusted were the following: distance to the collector, pumping speed, voltage and collection time, as mentioned in **Table 1**. The best electro-spinning conditions were as follows: DC 11 cm, VB 0.01 mL/h, V 14 kV, T 70 min and 16% PVA. Under different conditions drip was obtained on the collector, as shown in **Figure 2**.

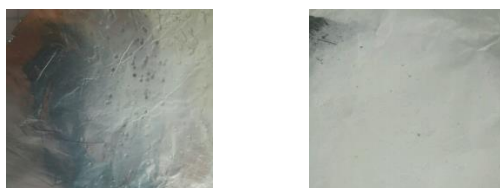


Figure 2. a). Drip on the aluminum collector plate for different conditions of: DC 11 cm, VB 0.01 mL/h, V 14 kV, T 70 min, b) Electro-spun nanofibers in the best conditions

3.3. Characterization of QMPM-PVA solutions with metal halides

Solutions with metal halides were synthesized with mentioned in the experimental section with QMPM. Subsequently, spectroscopic characterization was carried out in solution in the UV-Vis region, **Figure 3**. A significant electronic effect is observed in the electronic transitions assigned $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ (between 250-300 nm) of the chitosan, with respect to the compounds obtained, which suggests an interaction or formation of bonds between the polymer matrix and metal centers. In the case of manganese, the effect is significant since in a high spin configuration and 2+ oxidation state, there are no significant electronic transitions in the visible region, but there is a strong transfer of Metal-binding and Metal-binding loads. It is the same effect with the Ag^{1+} ion, which has no electronic transitions in the visible one, but a transfer effect in the UV region. On the other hand, since metal halide solution show counterions, such as chlorides easily displaced by coordinating groups, such as $-\text{OH}$ and $-\text{NH}_2$ present in the chitosan. The expected electronic transitions for a configuration are not observed in the visible region d^9 , d^7 for Cu^{2+} and Co^{2+} , which suggests that there is no coordination or little electronic effect, of the metal with respect to the polymer matrix.

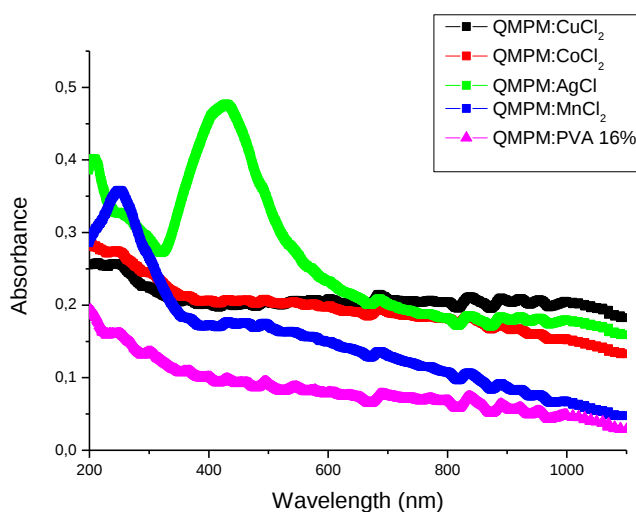


Figure 3. a). Electronic spectra of QMPM:PVA and metallic halides in the region UV- Vis

In the IR spectrum given the interaction of groups that promote hydrogen bonds in the chitosan and in the PVA, the displacement of the characteristic bands was not conclusive depending on the coordination, **Figure 4**. For copper and manganese, major shift in function of coordination to chitosan is observed. The vibration assigned to alcohol group $-\text{v} (-\text{OH})$ in 1088 cm^{-1} for chitosan don't showed big changes in comparison with carbonyl groups. This behavior suggested that the polymer interacts with metal ion by pair electron corresponding to oxygen carbonyl. Neither great interactions with the respective amine group were observed.

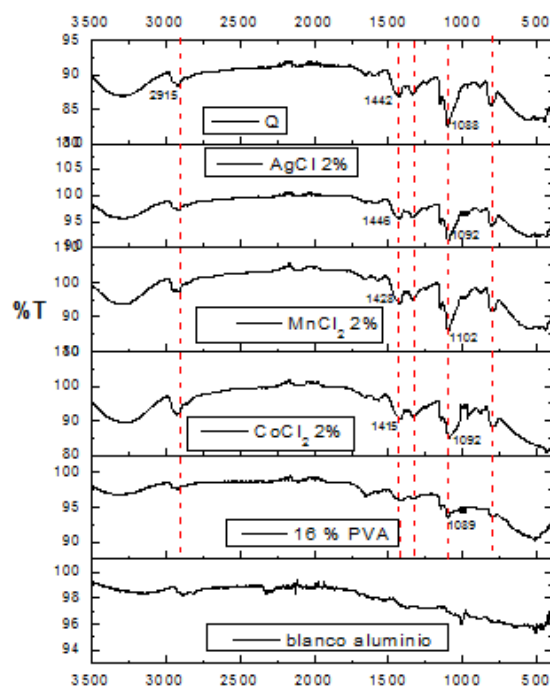


Figure 4. IR spectra for Q- PVA- Metal ion

For each case, the best conditions established of electro-spinning for aluminum were reported in **Table 5**, which there were no bubbles on the collecting plate. The necessary voltage decreases with the addition of the metal salt as expected. It also increase the pH value, which favors deprotonation of the acetyl group. This behavior is congruent with the effect of the metal ion on the vibration in carboxylic group.

Table 5. Optimal conditions in electrospun for Q-PVA- Metal ion.

Sample	Concentration (ppm)		Electrohilated Parameters	
Q-PVA	Q	84%	Colector distance	11 cm
			Variable voltage	19,5 kV
	PVA	16%	Time	40 min
			Humidity	50%
			Temperature	25,7 °C
			pH	5
Q-PVA CuCl2	Q	84%	Colector distance	11 cm
	PVA	16%	Variable voltage	19,4 kV
			Time	40 min
	CuCl2	2%	Humidity	54%
			Temperature	24,8 °C
			pH	6
Q-PVA CoCl ₂	Q	84%	Colector distance	11 cm
	PVA	16%	Variable voltage	18,4 kV
			Time	40 min
	CoCl ₂	2%	Humidity	52%
			Temperature	25,5 °C
			pH	6
	Q	84%	Colector distance	11 cm

Q-PVA MnCl ₂	PVA	16%		18,3 kV
			Time	40 min
	MnCl ₂	2%	Humidity	52%
			Temperature	26,3 °C
Q-PVA AgCl	Q	84%	pH	6
	PVA	16%	Colector distance	11 cm
			Variable voltage	18.2 kV
	AgCl	2%	Time	90 min
			Humidity	73%
			Temperature	27.5 °C
PVA	PVA	16%	pH	5.5
			Colector distance	11 cm
			Variable voltage	18 kV
			Time	20 min
			Humidity	70%
			Temperature	27.6 °C
			pH	5.9

* Pumping Speed constant 0,12 mL/h.

Finally, for these films were evaluated the antimicrobial properties. In the **Table 6**, is reported bacteriostatic effect for manganse(II) and silver(I), which shows high activity. For copper(II) and cobalt(II) the activity was not observed. These results affirm that the grater the interaction of the polymer matrix with metal ion, the greater the activity. For Chitosan- PVA as matrix without metal ion, no activity was observed. This is important, as it shows that electrospun films improve their activity with the presence of some coordination metals.

Table 6. Antimicrobial activity for Q-PVA- Metal ion

Sample		Concentration (ppm)	Bactericide	Bacteriostatic	% of inhibition*
Q-PVA-MnCO ₃	Q	20098,1	Active	-----	84,5%
	PVA	16%			
	MnCO ₃	2001			
Q-PVA-AgCl	Q	20098,1	Active	-----	58,9%
	PVA	16%			
	Ag	2009,5			

*Halo of inhibition sample / halo of antibiotic inhibition x100

Conclusions

The stabilization of the films by electro-spinning is mediated by variables such as: viscosity and voltage. For the samples under study, these variables showed a directly proportional behaviour. The addition of PVA to the chitosan achieves a decrease in the applied voltage as a condition in the optimization of the electrospinning process. The best interaction with water is shown at 16% PVA, because it increases the hydrophobic capacity of the film. Finally, coordination compounds between chitosan and metal manganese and copper salts are proposed through the electronic acetyl carboxy oxygen pairs. The biocidal properties are improved by the influence of the metal ion. However, it is necessary to continue with analyzes that allow determining the mechanism of action on microorganisms.

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